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Origin of Micromolecules

(Fox, Genaux)

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In the course of studies on the thermal copolymerization of cystine with other amino acids, control experiments have revealed that cystine under suitable conditions can form many other amino acids, aspartic acid being quantitatively prominent among these. Cystine, as one of the larger micromolecules, is a logical candidate for being a mother micromolecule. These results focus also on the importance of understanding the behavior of sulfur in the primitive earth.

Generation of Protein-like Macromolecules

(Fox, Harada)

Thermal proteinoids have been further characterized through improvements of the Akabori hydrazinolysis method. These studies have shown that proteinoids tend to have 2-4 C-termini per N-terminus. This indicates a degree of branching comparable to that found in proteins.

Krampitz and Knappen at the University of Bonn have published further on the nutritional quality of proteinoids in a paper titled, "Distribution of Radioactive Sulphur after Oral Administration to the Rat of a Water-insoluble Fraction of Sulphur-35-labelled Thermal Amino-Acid Copolymer" Nature 197, 289 (1963).

Catalytic Activity in Thermal Polyamino Acids

(Fox, Rohlfing, Hoagland)

The catalytic activity for p-nitrophenyl acetate of the proteinoids is found to a considerable degree in the thermal copolymers of a few amino acids

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such as aspartic acid and histidine. This is somewhat in contrast to copolymers made by Katchalski and co-workers through the Leuchs anhydrides. The tentative explanation of the difference is that the thermal process yields imide linkages, which have come into prominence as a site for catalytic activity in studies in many laboratories, but only in recent months. The thermal copolymers contain high proportions of this structure.

Many simple imides have been studied for the rate at which they are opened by alkali titration. Such studies have been aided by constant pH titration studies through use of the Titrigraph. The results can be related to the behavior of thermal copolymers of aspartic acid, and are of special interest in view of the suggestions recently made by others that aspartimide structures, perhaps transitionally, are parts of the active sites of many enzymes.

That the proteinoids are true catalysts has been verified by spectral and by titration studies. Acetate is both accepted and liberated by the polymers.

#### Binding of Hemes by Proteinoid

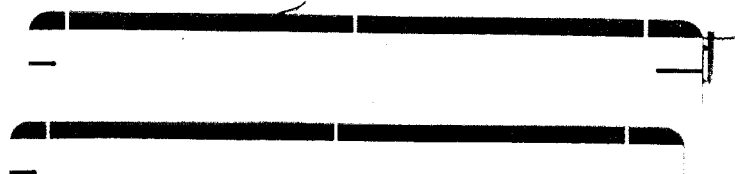
(Fox, Stewart, Mann)

Evidence has been obtained spectrally for the binding of hemes by proteinoid. This is being pursued systematically.

#### Synthesis of the Active Site of Hydrolytic Enzymes

(Fox, Hayakawa, Harada)

Many informal reports have been received of failure to synthesize the active site found to be present in many hydrolases,  $\alpha$ -L-aspartyl-L-seryl-glycine. This synthesis has now been accomplished in this laboratory. The tripeptide itself has no catalytic activity for the hydrolysis of p-nitrophenyl acetate. The tripeptide is being copolymerized into polymers with histidine.



### Generation of Polynucleotides

(Fox, Schwartz, Bradley)

By more careful fractionation than before, preparations of the thermal polymer of cytidylic acid are found to be attacked by ribonuclease. Substantial proportions of cytidylic acid can be recovered following hydrolysis of the polymer. Samples of these and other thermal polymers of mononucleotides have been sent to Dr. Marshall Nirenberg, who has found no coding power in them. Probably the molecular weight is yet too low.

G. Schramm has claimed that mononucleotides can be polymerized thermally by using an ethyl derivative of polyphosphoric acid. Except for replacing polyphosphoric acid by an ethyl derivative, the conditions reported by Schramm are identical to some of those reported earlier from this laboratory for the polymerization of amino acids. Many laboratories, including ours, have been unable to confirm Schramm's report. We have however made much progress with our own modification of polyphosphoric acid, again using conditions which are otherwise identical to those we have used earlier for polymerization of amino acids. Schramm's work as well as ours reemphasizes the importance of warm anhydrous systems.

### Simultaneous Polymerization of Leuchs Anhydrides of the Amino Acids Common to Protein

(Fox, Hayakawa, Windsor)

This intricate synthesis has been accomplished and simplified. The products are valuable for comparison with thermal proteinoids. The latter are already receiving intensive nutritional study at another laboratory (Bonn) but the polymers from Leuchs anhydrides have some unique advantages in this connection. The amino acids are nearly entirely L, and the quantitative profile of the hydrolyzate can be, and in one case has been, made virtually indistinguishable from the profile obtained from a natural protein.

The history of general knowledge of protein nutrition calls to mind that the early approaches to explaining protein nutrition in terms of amino acids rested on attempts to destroy in proteins one amino acid at a time. This approach proved to be so difficult that it was abandoned. Instead we have the monumental effort of W. C. Rose in which he assembled free amino acids and fed these. It is yet preferable to study the effects of individual amino acids from peptide combinations as pointed out by Rose (Nutrition Abstracts and Reviews, 1957), e.g., the free amino acids distort the caloric relationships. With the aid of both thermal and Leuchs proteinoids, it will now be possible to study such relationships in synthetic analogs of proteins. Already, individual amino acids such as phenylalanine, histidine, have been omitted from these polymers.

#### Extraterrestrial Macromolecular Sampler

(Fox, Windsor, with Hobby, Durrum)

It has been learned that when proteinoids are hydrolyzed for only 4 hrs. instead of the usual 12-24 hrs. (which would be an excessive period of time in a first Mars approach) the profile is very similar except for a complex peak at the histidine location. Also, useful results are obtained when the polyamino acid is first diluted with major amounts of washed Stromboli volcanic beach sand. The results are obtained through the use of ninhydrin which would not withstand the heat sterilization contemplated for the Mars vehicle. Experiments are under way to attempt to replace ninhydrin by other staining reagents.

#### Further Studies with Microspheres

(Fox, Yuyama, Fukushima)

With the partial exception of polyglucoses, no component of the reported models of steps in abiogenesis other than the thermal polyamino acids is a logical candidate for the origin of the cell as a unit. The gross structure of

cells as we know them depend predominantly upon protein. We cannot expect to find in micromolecules the properties already found to be associated with this model. Among the macromolecules, the nucleic acids are ruled out inasmuch as they do not contribute directly to the morphology of cells. Therefore, it is of special significance that a cell model arises so easily out of a model of primitive protein. Many of the associated properties are briefly described in the First Annual Report of this institute, 1 November 1962.

In the time-lapse sequence of that report can be seen spontaneous enlargement of a "bud" (yeast-like) on a microsphere.

Electron micrography now reveals for microspheres made in the presence of lecithin structural features of additional interest. Such pictures are planned for inclusion in the second annual report.

#### Catalytic Hydrolysis of ATP

(Fox, Wiggert)

The effects of various metal ions on the hydrolysis of ATP over various pH ranges has been studied. Preliminary evidence of activity of zinc complexes of proteinoid greater than that of the equivalent amount of zinc is at hand. Evidence that such activity can be incorporated into microspheres has been obtained, but this inclusion has not yet been accomplished in a reproducible way.

#### Reports by K. Bahadur

Dr. Krishna Bahadur of the Chemistry Department of the University of Allahabad was appointed in this laboratory on an annual basis but remained only 4 1/2 months. Six weeks after returning to Allahabad he sent to this laboratory a typescript describing units ("living particles") that would "grow, multiply, and metabolize". On one of the three sections of that paper, Bahadur placed as coauthor the name of S. W. Fox. This was strictly without permission and in flagrant disregard of the practice (known to Bahadur) in this laboratory of rigorous repetition by several workers of any salient advance before

publication. The data were furthermore fraudulent in several respects. Cables, letters, and the State Department have been employed in the attempt to get the name of S. W. Fox removed from the paper in question. In the course of all this, Bahadur made his claims to newspapers in India. Counterparts of the phenomena which he claims are found in the simple microspheres which have been carefully and extensively studied here; the entire set of relationships and the various data are documented.

#### Conference on Molecular Matrices for Prebiological Chemistry

The conference indicated is being arranged and will bring together in October at Wakulla Springs, Florida almost all of the leading workers in the field plus many of those who have written about the subject matter.

#### Theoretical Studies of the Atmosphere of Venus

##### Vertical Structure above the Cloud Surface of Venus

(Hess, Joern)

Numerical studies of this problem are being continued. The high-speed electronic computing is being done at Offut Air Force Base, Omaha. A large number of numerical results needed for the final computation have been generated. The machine program for the final computation has been written and is being tested.

##### Vertical Structure below the Cloud Surface of Venus

(Hess)

A theoretical model of the general circulation of Venus in the deep layers of the atmosphere has been formulated. The goal is to determine whether atmospheric transport of heat energy received from the sun at the high cloud layer can be transported downward by circulation processes. Such a result would explain the high temperatures at the solid planetary surface detected by radio means from Earth and from Mariner II.

Considerable difficulty has been experienced in getting meaningful numerical results on the University's IBM 709 computing machine. It has been found that specifications designed to prevent computational instability are conducive to the rapid onset of gravitational instability, and vice versa. This poses a serious problem since both instabilities must be avoided. Alternative approaches are being sought with the aid of a mathematical consultant from the Computing Center.

#### Physical Models of the Jupiter Atmosphere

(Hess, Hadlock)

Considerable progress has been made by the machine shop in construction of the rotating apparatus for simulation of circulation processes in planetary atmospheres. Completion of the basic apparatus is expected in a few weeks.

#### Instrumentation for Measurement of Water Vapor in the Atmosphere of Mars

(Hess, Cheng)

The first conception of a frost-point hygrometer to be dropped into the atmosphere of Mars has proved infeasible. An alternative approach which seems very promising has been conceived and the apparatus is being built.

#### Genetic Mechanisms

##### Tissue Culture

(Menzel)

Two clones of callus cells from Haplopappus gracilis have been grown on a simple mineral-sugar-vitamin-agar medium through 13 subcultures. One clone (2d) is a stable tetraploid ( $2n = 4x = 8$ ); the other (2e) contained a mixture of diploid and tetraploid cells through the 8th subculture but apparently only diploid cells in the 9th - 13th subcultures. Clone 2e occasionally organizes spontaneous meristems which give rise to short, histologically differentiated roots; clone 2d has never given rise to differentiated tissue. Six additional

clones are under observation (4th subculture).

Addition of indoleacetic acid and kinetin to agar medium results in faster initial growth of subcultures; this advantage disappears after the first week, as compared with controls. Other supplements tested had little effect. Since any desired amount of solid callus culture can now be produced at will, attention has turned to inducing dispersed growth in liquid medium in order to (1) control the rate and uniformity of cell division and (2) render individual cells more uniformly and immediately accessible to experimental manipulation.

Acceptable dispersion and growth rate have been obtained in some experiments, but results have been neither uniform nor predictable. Clones 2d and 2e have shown best growth in basal medium and addition alone and in combination of various supplements, including indoleacetic acid, kinetin, and coconut milk, recommended by other workers, has been either ineffective or toxic.

### Evolutionary Divergence of Chromosomes

(Menzel)

An  $F_1$  intergeneric hybrid, Lycopersicon esculentum X Solanum lycopersicon, was selected for study since it fulfills criteria for a hybrid combining partially nonhomologous genomes. Earlier study of meiotic chromosome behavior had shown no evidence of heterozygosity for small linear rearrangements, and the two genomes appeared identical and capable of complete normal synapsis except for 4 out of 24 heterochromatic segments which differed in length; meiotic irregularities were due mainly to faulty distribution of chiasmata rather than to failure of synapsis or reduction of chiasma frequency.

Pachytene and metaphase I in the corresponding allotetraploid were studied in detail by conventional cytological methods and it was found that synapsis is almost completely preferential in the tetraploid. Thus a pair of partial homologues,  $S_1$  and  $L_1$ , synapse perfectly in the  $F_1$  but only  $S_1S_1$  and  $L_1L_1$  pairs are formed in the allotetraploid. Hence chromosomes  $L_1$  and  $S_1$



must differ in some manner which enables each to "prefer" the more nearly similar of two possible kinds of partner at synapsis.

Two further questions are being asked of this system:

1. Is the difference between L and S chromosomes one of timing, e.g., in DNA replication? Attempts are being made to answer this question by labelling chromosomes of  $F_1$  pollen mother cell nuclei with  $H^3$ -thymidine. Equipment, supplies and prerequisite technical skill for making autoradiographs have been acquired. Since controlled-environment facilities for growing plants are not available, the crucial problem of introducing labelled DNA precursor into the pollen mother cells cannot be tackled until the hybrid plants enter another cycle of flower formation (in October).

2. Is the apparent intimate synapsis of L and S chromosomes "true" synapsis, e.g., is it accompanied by the formation of a "synaptanmental complex" such as Moses, Ris and others have reported in electron micrographs of synapsed chromosomes in various species? Attempts are in progress to compare thin sections of pollen mother cell nuclei at pachytene in the  $F_1$  and the tomato parent under the electron microscope, with the advice and assistance of Mrs. Charlotte Dockery, but many technical problems remain to be solved.

#### Comparative Studies on Reproductive Physiology

(Metz, Mann, Lutwak-Mann, Austin, Shivers, Franklin, Stern, Hampson)

Organisms ranging from mammals to protozoa were studied by this group of workers.

Among mammals, a series of experiments was conducted on fertilization of rabbit eggs in vitro and on the probable factors influencing capacitation of sperm in vitro.

Studies on the blastocyst fluid of the opossum revealed an unusually high (2%) glucose content.

Comparisons have been made by immunoelectrophoresis of antigenic components found in pig serum, follicular fluid, and uterine secretions by preparing antibodies against these substances in the rabbit. It was found that each of these fluids contained from twelve to fifteen antigenic components. By appropriate adsorption arrangements it was found that most of these antigens were shared among all three fluids. In addition to these shared antigens, pig serum was found to contain two, follicular fluid one, and uterine secretion four specific components.

A series of biochemical and morphological studies were made on the male accessory organs and on the spermatozoa of a variety of lower vertebrates.

For the study of sperm storage sites in the snake female genital tract, specimens have been collected from various parts of the tract in 14 rattlesnakes, 10 king snakes, and 11 racers. These specimens have been processed for histological examination; scrutiny of the slides will be done by Dr. Austin on return to Cambridge, England.

Detailed observations have been made on the morphology of spermatozoa in species of snakes (Crotalus, Lampropeltis, Coluber, Elapis, Drymarchon and a South American boa constrictor) and amphibians (Bufo, Rana, Amphiuma, Siren and Pseudobranchius). The observations were mostly by phase contrast microscopy, but some studies were made of stained preparations and whole-mounts for electron microscopy. Blocks have also been prepared of Crotalus sperms for electron microscopy of ultra-thin sections.

In addition it was found that snake sperm metabolism resembles that of birds and mammals to the extent of consuming fructose anaerobically.

Another project has been the study of the role in fertilization of jelly coat material of the frog egg. It was demonstrated that sperm which had been in contact with the jelly were capable of fertilizing jellyless, coelomic eggs. Since jellyless eggs are not normally fertilizable, it was concluded that

the sperm undergoes some morphological and / or physiological change in the presence of jelly which allows them to unite with the egg in successful fertilization. This change in the frog sperm has been compared to the "sperm capacitation" phenomena which takes place in mammalian sperm in the female reproductive tract. The time required for "capacitation" in the frog sperm is 10 to 15 minutes.

An extensive electron microscope study of sperm penetration into sea urchin oocytes and egg structure was carried on. The evidence supports the sperm-egg membrane fusion hypothesis of sperm penetration. Additional conjugations of ferritin to antibody for sperm antigen localization were obtained.

An investigation was made on aspects of fertilization in the Mesozoon Dicyema aegira. Material for this work was made available by Dr. R. B. Short.

Preliminary tests have been made on the effect of the chelating agent EDTA upon conjugation in Paramecium aurelia.